FORM PTO-1390 (Modified) 1-08 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: AAT-11612

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/269845

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB97/02721	October 3, 1997	October 3, 1996

TITLE OF INVENTION: COLOSTRININ, AND USES THEREOF

APPLICANT(S) FOR DO/EO/US: Marin Janusz, Jozef Lisowski and Anna Dubowska-Inglot

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [XX] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [XX] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [XX] have not been made and will not be made. *PRELIMINARY AMENDMENT ENCLOSED*
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

EL069441064US

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/GB97/02721	AAT-11612

17. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1070.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $840.00 ~~$930.00~~		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $790.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $720.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $98.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$
Total claims	42 -20 =	22	x ~~$22.00~~ 18.00	$ 396.00
Independent claims	18 -3 =	15	x ~~$82.00~~ 78.00	$ 1,170.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
TOTAL OF ABOVE CALCULATIONS =				$
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). +				$
SUBTOTAL =				$ 2,406.00
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$
TOTAL FEES ENCLOSED =				$ 2,406.00
			Amount to be refunded:	$
			charged:	$

a. ☒ A check in the amount of $ 2,406.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 18-0160 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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32,119
REGISTRATION NUMBER

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	
Marin Janusz, Józef Lisowski and Anna Dubowska-Inglot	Group Art Unit
	Examiner:
International Application No. PCT/GB97/02721	Atty. Docket No.: AAT/11612
International Filing Date October 3, 1997	
For: COLOSTRININ, AND USES THEREOF	

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sir:

Prior to calculating the filing fee, and prior to examining the above noted application (U.S. National Phase), please amend such application as follows:

Please cancel claims 1, 2, 8, 23, 37, 39, 42, 43, 45, 48, 49 and 50 without prejudice.

Please cancel/delete the second occurrence of claim 44 on page 26, lines 17-18.

Please note that there is no claim 38.

Please amend claims 10, 11, 12, 20-22, 26, 27, 30 and 33-36 as follows:

10. (Amended) Colostrinin according to claim 9 [or 10], for use in the treatment of diseases with a bacterial and viral aetiology, and/or for use in the treatment of acquired immunological deficiencies.

11. (Amended) Colostrinin according to claim 9[, 10 or 11], for use in the treatment of chronic bacterial and/or viral infections.

12. (Amended) Colostrinin [according to any preceding claim,] wherein said Colostrinin is derived from a non-ovine source.

20. (Amended) The use according to claim 18 [or 19], or the treatment of diseases with a bacterial and viral aetiology, and/or for use in the treatment of acquired immunological deficiencies.

21. (Amended) The use according to claim 18[, 19 or 20], for the treatment of bacterial and/or viral infections.

22. (Amended) The use according to claim [any one of claims] 18 [to 21], wherein said Colostrinin is non-ovine Colostrinin.

26. (Amended) A method according to claim 23[, 24 or 25], wherein the Colostrinin is non-ovine Colostrinin.

27. (Amended) A method according to claim [any one of claims 23 to] 26, [comprising] wherein said predetermined amount of Colostrinin is in the range of about 25 to 2000 micrograms.

30. (Amended) A method according to claim 28 [or 29], wherein the cycle is repeated at least once.

33. (Amended) A composition according to claim 31 [or 32], in a form suitable for injection.

34. (Amended) A composition according to claim 31 [or 32], in a form suitable for absorption through the mucosa of the oral/nasopharyngeal cavity and/or in a form suitable for absorption in the alimentary canal.

35. (Amended) A composition according to claim [any one of claims] 21 [to 34], in the form of a tablet, lozenge, gel, patch or plaster.

36. (Amended) A composition according to claim [any one of claims] 31 [to 35], comprising 25 to 1000 micrograms of Colostrinin.

Comments/Remarks

Pursuant to the above amendment, claims 3-7, 9-36, 40, 41, 44, 46, 47 and 51-55 are pending in the application.

Respectfully submitted,

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COLOSTRININ AND USES THEREOF

The present invention relates to Colostrinin, and to its use as a medicament.

Colostrum is the thick, yellowish fluid produced by a mammalian mother's breasts during the first few days after childbirth. It is replaced by mature breast milk about four to five days after birth. Compared with mature breast milk, colostrum contains low sugar. However, colostrum is richer in lipids, protein, mineral salts, vitamins and immuno-globulin. It also contains various floating cells such as granular and stromal cells, neutrophils, monocyte/macrophages and lymphocytes and includes growth factors, hormones and cytokines.

Various factors have been isolated and characterised from mammalian colostrum. In 1974, Janusz et al (FEBS Lett., 49, 276-279) isolated a proline-rich polypeptide (PRP) from ovine colostrum. The contents of this reference are incorporated herein by reference. It has since been discovered that mammals other than sheep have analogues of PRP as a component of their colostrum. PRP has since been called Colostrinin and is tentatively identified as a new class of cytokine.

Janusz et al in "Proline-Rich Polypeptide (PRP) - an Immunomodulatory Peptide from Ovine Colostrum" (Archivum Immunologiae et Therapiae Experimentalis, 1993, 41, 275-279) mentioned that PRP from ovine colostrum has immunotropic activity in mice. However, there was no suggestion in this document that the PRP would have any therapeutic effect on any other animal. It will be appreciated that the fact that a composition has a therapeutic effect on mice cannot be taken to suggest that there will be a therapeutic effect on any other animal.

We have now found that Colostrinin has a number of previously unknown therapeutic effects. More particularly, we have found that Colostrinin provides an immunotropic action and provides a psychotropic

action.

According to one aspect of the invention we provide Colostrinin for use as a medicament. The Colostrinin may be used as a medicament for non-rodent mammals; we have found that Colostrinin is especially useful as a medicament for the treatment of humans.

According to another aspect of the invention there is provided the use of Colostrinin in the manufacture of a medicament for treating disorders of the central nervous system or disorders of the immune system.

In one advantageous embodiment of the invention, the Colostrinin is for use in the treatment of disorders of the central nervous system, particularly chronic disorders of the central nervous system. The disorders of the central nervous system that may be treated with Colostrinin include neurological disorders and mental disorders.

Examples of neurological disorders that can, with advantage, be treated by Colostrinin include dementia, and also disorders that cause dementia, such as neurodegenerative disorders. Neurodegenerative disorders include, for example, senile dementia and motor neurone disease; Parkinson's disease is an example of a motor neurone disease that can be treated with Colostrinin. Colostrinin has been found particularly effective in the treatment of the neurodegenerative disease known as Alzheimer's disease.

Examples of mental disorders that can, with advantage, be treated by Colostrinin include psychosis and neurosis. For example, the Colostrinin may be used to treat emotional disturbances, especially the emotional disturbances of psychiatric patients in a state of depression - the use of Colostrinin has been found to help the patient with an improvement in feelings of wellbeing and with mood stabilisation. The Colostrinin may also be used as an auxiliary withdrawal treatment for drug addicts, after a period of detoxification, and in persons dependent on stimulants.

In another advantageous embodiment of the invention, the Colostrinin is for use in the treatment of disorders of the immune system,

particularly chronic disorders of the immune system. Thus, we have found that Colostrinin can be used in the treatment of disease requiring immuno-modulation. In particular, we have found that Colostrinin is useful for treating such disorders in non-rodent animals, including humans. Colostrinin is useful in the treatment of a variety of diseases with an immunological and infectious basis. For example, the Colostrinin can be used to treat chronic diseases with a bacterial and viral aetiology, and to treat acquired immunological deficiencies that have developed, for example, after chemotherapy or radiotherapy of neoplasms. The invention is particularly useful for treating chronic bacterial and viral infections requiring non-specific immunostimulation and immunocorrection.

In general, a chronic disorder is a disorder that has persisted for a long time, usually at least one week, more usually at least one month, and often at least 3 months or at least 6 months.

It is a feature of the present invention to use Colostrinin for improving the development of the immune system of a new born child. It is a further feature of the invention to use Colostrinin to correct immunological deficiencies in a child. These uses of the Colostrinin may be particularly applicable to babies or children who have been deprived of colostrum. This may occur, for example, in babies and children who were not breast fed from birth; the artificial feed that such babies and children would have been given does not contain Colostrinin.

According to another aspect of the invention, we provide the use of Colostrinin as a dietary supplement. Colostrinin is particularly advantageously used as a dietary supplement for babies and young children to correct deficiencies in the development of their immune system. As noted above, such deficiencies would arise in babies and children who had not been breast fed from birth. The Colostrinin may also be used as a dietary supplement for adults who have been subjected to chemotherapy, or have suffered from cahexia, or weight loss due to chronic disease. In an aspect of

the invention, we provide a dietary supplement comprising an orally ingestible combination of Colostrinin in combination with a physiologically acceptable carrier. The dietary supplement may be provided in liquid or solid form; the dietary supplement may suitably be provided in the form of a tablet.

In accordance with the invention, the Colostrinin may be administered prophylactically in order to help to prevent the development of disorders of the central nervous system and the immune system.

The Colostrinin used in the aspects of the invention described above may be ovine Colostrinin, or it may be non-ovine Colostrinin. Non-ovine Colostrinin may be derived from the colostrum of, for example, humans, cows, horses, goats, pigs, yaks, llamas and asses. The colostrum will normally be present in the beestings of these animals for 1 to 4 days after parturition.

The term "Colostrinin", as used herein refers to a polypeptide which, in its natural form, is obtained from mammalian colostrum. Colostrinin is sometimes known as "colostrinine", and has the following properties:

(i) it has a molecular weight in the range 16,000 to 26,000 Daltons;

(ii) it is a dimer or trimer of sub-units each sub-unit having a molecular weight in the range 5,000 to 10,000 Daltons, preferably 6,000 Daltons;

(iii) it contains proline, and the amount of proline is greater than the amount of any other single amino acid.

We have also found that the Colostrinin, and also the sub-units making up the Colostrinin, are non-polar.

The molecular weight can be determined by electrophoresis in the presence of SDS; the presence of the dimer or trimer can be shown by the same technique. It can be shown that the bonds between the sub-units are non-covalent by electrophoresis in reduced and non-reduced conditions. The presence of proline can be established by conventional amino acid analysis.

The non-polarity can be demonstrated by chromatography in non-polar conditions.

The Colostrinin used in the present invention may be ovine-Colostrinin or non-ovine Colostrinin. Ovine Colostrinin has a molecular weight of about 18,000 Daltons, is made up of three non-covalently linked sub-units each having a molecular weight of about 6,000 Daltons and includes about 22 wt% proline. The amino-acid composition is made up of the following number of residues per sub-unit: lysine - 2, histidine - 1, arginine - 0, aspartic acid - 2, threonine - 4, serine - 3, glutamic acid - 6, proline - 11, glycine - 2, alanine - 0, valine - 5, methionine - 2, isoleucine - 2, leucine - 6, tyrosine - 1, phenylalanine - 3 and cysteine - 0.

As noted above, in its natural form Colostrinin is derived from mammalian colostrum. Colostrinin can be derived from mammalian colostrum by removing the lipids and the majority of the proteins from the colostrum. Broadly, Colostrinin can be obtained from the beestings of, for example, large farm animals using column chromatography techniques and other biochemical techniques, or can be obtained by genetic engineering techniques.

More particularly, Colostrinin may be isolated from mammalian colostrum by using the following steps:

(i) Removing lipids, for example by centrifugation;

(ii) Removing proteins such as casein, for example, by lowering pH;

(iii) separating Colostrinin bound to immunoglobulin, for example by:

(a) Processing the whey formed after the removal of lipids and proteins by ion exchange chromatography; and

(b) Eluting with phosphate buffered saline and collecting a fraction containing Colostrinin bound to immunoglobulin, for example IgG2 in sheep;

(iv) Separating Colostrinin from the immunoglobulin, for example by sieving chromatography; and

(v) Further purifying the Colostrinin, preferably by:

(a) De-salting the fraction below 30,000 Daltons molecular weight; and

(b) Introducing antibodies to immunoglobulins and thereby remove this class of proteins to obtain the final product.

Whilst the above definition relates to naturally occurring mammalian Colostrinin, the term Colostrinin as herein used also includes analogues and fragments thereof having substantially the same biological activity, and mammalian Colostrinin, analogues thereof and fragments thereof produced by recombinant DNA technology. Colostrinin as used herein also includes biologically active polypeptides of substantially the same composition as natural Colostrinin, which have been made by polypeptide synthesis.

In a further aspect of the present invention there is provided a method of treating disorders of the central nervous system or of the immune system using Colostrinin. The disorders that can, with advantage, be treated using the method according to the invention are described above. In a preferred embodiment the Colostrinin is administered to a patient for a first period at about 1 to 2 therapeutic units daily, followed by a second period when no Colostrinin is administered. The first period is preferably about 2 to 4 weeks, more preferably about 3 weeks; and the second period is preferably about 2 to 5 weeks, more preferably about 4 weeks. This cycle is preferably repeated at least once, and is more preferably repeated more than once.

The therapeutic unit for use in methods of the invention is preferably in the range 25 to 1,000 micrograms of Colostrinin, most preferably 50 to 100 micrograms.

The Colostrinin may be formulated for administration in any

suitable form. For example, is may be formulated for oral, rectal or parenteral administration. More specifically, the Colostrinin may be formulated for administration by injection, or, preferably, in a form suitable for absorption through the mucosa of the oral/nasopharyngeal cavity, from the alimentary canal or any other mucosal surface. The oral formulations may be provided in a form for swallowing; or, preferably, in a form for dissolving in the saliva, whereby the formulation can be absorbed in the mucous membranes of the oral/nasopharyngeal cavity. The oral formulations may be in the form of a tablet for oral administration, lozenges (i.e. a sweet-like tablet in a form suitable to be retained in the mouth and sucked), adhesive gels for rubbing into the gum. The Colostrinin may be formulated as an adhesive plaster or patch, which may be applied to the gums. The Colostrinin may also be formulated for application to mucous-membranes of the genito-urinary organs.

Whilst it would, of course, be possible to administer the Colostrinin in the form of whole colostrum, this is not preferred, because whole colostrum has an unpleasant taste, and is difficult to store.

Colostrinin for use in the present invention may be obtained from any mammal, including human sources or animals such as cows, horses, goats, pigs, yaks, sheep, llamas or asses, camels etc.

Tests on Colostrinin were performed using ovine and human Colostrinin. Ovine Colostrinin is marketed under the trade mark Colostrinin™.

During tests on experimental animals it was found that Colostrinin is characterized by immunotropic action, both *in vivo* and *in vitro*, based on the properties of modulation, development of differentiation and maturation, of thymocytes to active T cells and on the stimulation or inhibition of an immunological response, and on induction of the expression of various surface markers on the thymocytes. In intraperitoneal administration in mice, Colostrinin inhibits the development of haemolytic anaemia in mice of the NZB line, inhibit the growth of sarcoma 180 in mice, and in mice exposed to

gamma radiation it protects the animals against radiation sickness. Toxicological studies on mice showed, both after oral and parenteral administration, a very low toxicity, as LD50 is above 1.25 g/kg of body weight. Colostrinin also exhibits capacity to stimulate the growth, maturation and differentiation of immunologically active cells both in humans and in experimental animals. In cultures of lymphocytes of human peripheral blood (including cultures of lymphocytes isolated from the cord blood) Colostrinin is characterized in that it stimulates the production of cytokines, especially gamma interferon (IFN-γ), tumour necrosis factor (TNF-α), interleukins (e.g. IL-6 and IL-10) and various growth factors. The cytokines produced are determined quantitatively by known methods.

In natural conditions analogues of ovine Colostrinin but possessing the biochemical properties thereof are present in human colostrum and in the beestings of all mammals, and especially large farm animals such as cows, horses, goats, pigs, yaks, sheep, llamas or asses, camels etc. for 1-4 days after parturition. The Colostrinin enters the body of the newborn animals during sucking and swallowing of its mother's colostrum. Owing to the low molecular weight it is possible for Colostrinin to act on the mucosa of the oral/nasopharyngeal cavity via cell receptors, and even as a result of ordinary diffusion. Because the mucosa and epithelium of the upper part of the digestive tract contain receptors for certain immunomodulators, it has been shown in investigations that the Colostrinin can be administered in the form of tablets and sublingual tablets for sucking, tablets and capsules for swallowing, in the form of adhesive gels, and in the form of adhesive plasters for fastening to the gums. Colostrinin can also be applied to the mucous membranes of genito-urinary organs.

Scientific studies aiming to elucidate the biochemical activity induced by Colostrinin also revealed the existence of similar biological activity when using human colostrum, collected from women in the period of 1-7 days after parturition. The method used for isolating Colostrinin of human

origin was analogous to the methods of obtaining it from the beestings of farm animals, and especially from sheep beestings. It was found that the colostrum secreted by the mammary gland of women from 1-7 days after parturition contains the polypeptide Colostrinin, the amount of which depends on lactation and is optimum between 2-3 days, when about 300 mg of it is detected in 1 litre of colostrum. This quantity of human Colostrinin is within the range seen in ovine colostrum. It was demonstrated that human Colostrinin has many biological properties similar to, for example, the sheep analogue. It was found that Colostrinin originating from human colostrum stimulates the lymphocytes present in the colostrum, and cord blood lymphocytes, to secrete cytokines, including mainly interferon (IFN-γ), tumour necrosis factor TNF-α, interleukins (IL-10 and IL-6) and other cytokines. These cytokines are involved in humoral and cellular immune reactions.

These biological activities of the Colostrinin both of human and of animal origin were determined by the method known from a Polish patent (PL 170592 B1) and from European patent no. EP-B-0609225, pt.: Testing Immunology.

In studies on human volunteers it was shown that after administration of tablets for sucking, containing at least 25 µg of Colostrinin at doses of at least one daily, for a period of about 3 weeks, no adverse reactions were observed; on the other hand it brings about a state of hyporeactivity to induction of interferon IFN-γ and partially also of tumour necrosis factor TNF-α. After discontinuing administration of the Colostrinin, the state of hyporeactivity returns to normal. The reactions obtained are the result of involvement of cytokines produced by Th1 and Th2 lymphocytes and by helper cells.

During clinical studies it was shown in addition that Colostrinin has an immunomodulatory effect and an effect on the central nervous system, which can be utilized prophylactically and therapeutically in the following diseases, e.g. for preventing occurrence of, progression of and for remission of

Alzheimer's disease, in senile dementia of some other origin and in the treatment of chronic diseases with an immunologic and infectious basis.

Instead of Colostrinin, it is possible to use a nonapeptide designated NP having the following composition and amino acid sequence: Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro. NP can be obtained from Colostrinin by digesting with chymotrypsin and isolation by column chromatography, or by means of chemical synthesis. NP is an example of a useful fragment of Colostrinin. It has been found to have similar biological effects to Colostrinin. and, in particular, is useful for treating the chronic immune and central nervous system disorders described above, especially Alzheimer's disease. NP may be used to treat any of disorders described above. It may also be used in a dietary supplement. It may be formulated in the same way as Colostrinin. NP is useful for the treatment of mammals, especially humans.

In order that the invention may be more fully understood, reference will now be made to the accompanying Examples by way of illustration only.

Example I

In ovine Colostrinin with molecular weight of 18 000 Daltons, constructed from three non-covalently linked subunits with molecular weight of 6 000 Daltons, each sub-unit had the following amino-acid composition:

Amino Acid	No. of residues per subunit
Lysine	2
Histidine	1
Arginine	0
Aspartic acid	2
Threonine	4
Serine	3
Glutamic acid	6
Proline	11
Glycine	2
Alanine	0
Valine	5
Methionine	2
Isoleucine	6
Tyrosine	1
Phenylalanine	3
Cysteine	0

The Colostrinin was obtained from sheep beestings, taken after parturition up to 24 hours after commencement of lactation. The material was centrifuged at 35 000 x g in order to remove fats and part of the casein. The Ig fraction obtained was applied to a DEAE-Cellulose column, in order to isolate immunoglobulin IgG2 complexed with the polypeptide Colostrinin, and upon elution from the column the Colostrinin was separated from the IgG2 by means of column chromatography on Sephadex G-100 and was chromatographed again on Sephadex G-75. Then a fraction of this polypeptide was applied to a column of Sepharose conjugated with anti-bodies

against sheep IgG2 to remove traces of IgG2 contamination. The preparation obtained was desalinated by means of Sephadex G-25 and lyophilized, after which it was stored at a temperature of +4°C or -20°C.

The Colostrinin thus obtained has been designated by the trade mark Colostrinin™.

In polyacrylamide gel electrophoresis in the presence of SDS, a strong band was observed, corresponding to molecular weight of 5 800 Daltons, and two weak bands corresponding to molecular weight of 12 400 Daltons and 18 200 Daltons.

Example II

The nonapeptide NP having the composition and amino acid sequence Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro was obtained from the Colostrinin made in Example I.

50 mg of the Colostrinin is digested by means of 10 activity units of the proteolytic enzyme chymotrypsin, for 20 hours at a temperature of 30°C. Isolation from the product of digestion was carried out by means of at least one cycle of column chromatography using Sephadex G-10. The preparation obtained was lyophilised, and was then stored at a temperature of +4°C or -20°C. The isolated nonapeptide was isolated by means of determination of the N-terminal amino acid.

Example III

Dosage unit in the form of a tablet for sucking, with the composition:

Active ingredient:	Colostrinin® polypeptide obtained according to Example 1	0.0001 g
Stabilizer:	Albumin, free from impurities, mainly I.P.S.	0.000135 g

Lubricant/binder:	Magnesium stearate	0.003 g
Carrier:	Mannitol	0.15 g (0.1497 g)

The above components were suspended in 0.0001 M NaCl. Tablets for sucking are primarily intended for treating early and late stages of dementia, including Alzheimer's disease, and various stages of senile dementia, for treating chronic bacterial and viral infections, requiring non-specific immunostimulation and immunocorrection and acquired immunologic deficiencies caused by various agents. In addition it is used in emotional disturbances especially in states of depression in psychiatric patients to improve the general feeling of well being and for mood stabilization, and in auxiliary withdrawal treatment of drug addicts, after a period of detoxification and in persons dependent on stimulants. Colostrinin™ can also be used in the newborn and in young children for correcting nutritional deficiencies connected with artificial feeding.

Example IV

Preparation in the form of gel for application to mucous membranes with the composition:

Active ingredient:	Colostrinin™ polypeptide obtained according to Example I
Stabilizer	Albumin
Gel carrier:	Orabase-Plain® containing pectin, gelatin, sodium salt of carboxymethylcellulose and hydrocarbon gel

0.0003 g of the polypeptide Colostrinin™ and 0.0015 g of

albumin were used per 1 ml of gel carrier. The dosage unit thus formulated is primarily intended for cyclic treatment of bacterial and viral infections of the oral cavity and upper respiratory tract.

Example V

Preparation in the form of intramuscular, subcutaneous or intravenous injections.

Active ingredient:	Colostrinin™ polypeptide obtained according to Example I
Stabilizer:	Human albumin, free from impurities
Carrier:	Sterile, none pyrogenic water

0.0003 g of Colostrinin™ polypeptide, 0.0015 g of albumin and, as antibacterial agent, 0.001% of Merthiolate, i.e. sodium salt of ethylmercurithiosalicylic acid, were used per 1 ml ampoule. The dosage unit thus obtained is primarily intended for cyclic treatment of bacterial and viral infections.

Example VI

Preparation in the form of intramuscular, subcutaneous or intravenous injections.

Active ingredient:	NP obtained according to Example II
Stabilizer:	Human albumin, free from impurities
Carrier:	Sterile, none pyrogenic water

0.0003 g of NP, 0.0015 g of albumin and, as antibacterial agent, 0.001% of Merthiolate, i.e. sodium salt of ethylmercurithiosalicylic acid, were used per 1 ml ampoule. The dosage unit thus obtained is primarily intended for cyclic treatment of bacterial and viral infections.

Example VII

Induction of cytokines by the polypeptide Colostrinin™ *in vitro* on blood taken from healthy and sick volunteers taking the pharmaceutical in the form stated in Example III, in cyclic treatment, is carried out as follows.

Whole blood, containing 10 units per 1 ml of heparin without preservatives is diluted at 1:10 ratio in RPMI 1640 culture medium. Incubation is conducted in the same culture medium without inducers (negative control), or in the presence of 1-100 µg/ml of the polypeptide Colostrinin™ or in the presence of 2 µg/ml blood of phytohaemagglutinin (PHA) and 2 µg/ml blood of lipopolysaccharide (LPS), at a temperature of 37°C, in an atmosphere of 5% carbon dioxide, for 20 hours. Samples with mixture of PHA and LPS are the positive control, as they can stimulate the maximum quantities of cytokines. The test results are presented in the Tables 1 and 2. They show that Colostrinin™ at concentrations of 1-100 µg/ml stimulates the production of cytokines in a dose-dependent fashion. Relative to the negative control (without inducers) these results are statistically significant ($p < 0.0001$). Patients with Alzheimer's disease exhibit diminished capacity for production of IFN and to a lesser extent also TNF.

Table 1

Induction of cytokines by Colostrinin™ (series A 1993) from sheep in cultures of lymphocytes present in whole blood of healthy volunteers or of patients with Alzheimer's disease.

Blood donors	Inducers	Dose (µg/ml)	Cytokines (unit/ml ± SD)	
			IFN	TNF
Healthy volunteers	Colostrinin™	100	344 ± 254	670 ± 560
	Colostrinin™	10	50 ± 59	316 ± 371
	PHA + LPS	2 + 2	171 ± 162	521 ± 447
	Control	-	9 ± 21	19 ± 26
Patients with Alzheimer's disease	Colostrinin™	100	21 ± 14	249 ± 187
	Colostrinin™	10	15 ± 9	182 ± 158
	PHA + LPS	2 + 2	117 ± 76	397 ± 252
	Control	-	2 ± 3	18 ± 26

The group of healthy volunteers (22 people) was heterogeneous (age range 20-64 years). The group of patients with Alzheimer's disease (50 people) was also heterogeneous (age 63 ± 7.5 years). Despite the high standard deviation (± SD) the differences between the controls without inducers (absent) and Colostrinin™ were statistically significant ($p < 0.001$).

Table 2

Examples of stimulation of the production of interferon (IFN) by Colostrinin™ (series A 1993) from sheep in experiments using whole blood of healthy volunteers and patients with various diseases.

Blood donors (diagnosis)	Inducer	Dose (µg/ml)	Titres of IFN determined by	
			Antivirus biotest units/ml	ELISA IFN-γ pg/ml
Z.B. healthy young soldier	Colostrinin™	100	300	2920
	Colostrinin™	10	300	3402
	Colostrinin™	1	100	1413
	PHA + LPS	2 + 2	200	3308
	Control	-	<3	24
C.D. healthy young soldier	Colostrinin™	100	600	3941
	Colostrinin™	10	200	3778
	Colostrinin™	1	100	2690
	PHA + LPS	2 + 2	600	4631
	Control	-	5	55
M.J. Alzheimer's disease	Colostrinin™	100	40	400
	Colostrinin™	10	20	427
	PHA + LPS	2 + 2	300	3757
	Control	-	3	46
S.E. schizo-phrenia	Colostrinin™	100	6	29
	Colostrinin™	10	6	29
	PHA + LPS	2 + 2	30	243
	Control	-	3	19
F.W. breast cancer	Colostrinin™	100	70	523
	Colostrinin™	10	50	307
	PHA + LPS	2 + 2	600	2833
	Control	-	40	150

NOTE: the biotest for presence of IFN measures the levels of various types of interferons, whereas the ELISA test only determines the level of immunoactive IFN-γ.

Example VIII

Cord blood obtained from the Gynaecological-Obstetric Department of the Specialist District Hospital in Wroclaw. Induction of cytokines by Colostrinin™ *in vitro* in lymphocytes of cord blood taken 4-6 hours after parturition is effected in the following way.

The lymphocytes are isolated using a Ficoll-Paque® gradient containing 5.7 g of the component Ficoll 400 and 9.0 g of the component Diatrizoate Sodium per 100 ml. The lymphocytes isolated are suspended in RPMI-1640 culture medium at density of 2×10^6 lymphocyte cells per 1 ml of culture medium. Colostrinin™ at concentration of 1-20 μg/ml or phytohaemagglutinin at concentration of 10 μg/ml is added to the lymphocyte suspension. The culture is incubated for 20 hours at a temperature of 37°C. Then the level of cytokines in the culture fluids is determined by biological methods. A typical example is given in Table 3. Colostrinin™ at concentration of 1-100 μg/ml has capacity for stimulation of cytokines (IFN and TNF) similar to that exhibited by the classical IFN-γ inducer - phytohaemagglutinin.

Table 3

Induction of cytokines by Colostrinin™ (series A 1996) in a culture of lymphocytes isolated from cord blood (CBL).

N	Inducer	Dose (μg/ml)	Cytokines (unit/ml ± SD)	
			IFN	TNF
32	Colostrinin™	20	89 ± 79	59 ± 41
39	Colostrinin™	10	78 ± 80	37 ± 35
17	Colostrinin™	1	38 ± 66	16 ± 17
50	PHA	10	75 ± 66	83 ± 69
50	control	-	3 ± 3	3 ± 4

N - number of CBL samples investigated

In the Student t test the probability (p) of significance of the difference Colostrinin™

- "absent", both for IFN and TNF, is $p < 0.0001$.

Because the cord blood is rich in immature stem cells, which are capable of reproduction of haemopoietic cells and of various immunologically-active cells, the result obtained shows that Colostrinin™ greatly accelerates the maturation of stem cells. The results obtained show that it is possible to use Colostrinin™ for treating various types of immune deficiencies and for stimulating the haemopoietic system, e.g. after injuries, infections, chemotherapy and radiotherapy. In biomedical studies, substances of natural origin with similar action are very seldom encountered.

Example IX

The method of treatment of disorders of the central nervous

system was investigated on a group of volunteer patients in the early and moderate stages of Alzheimer's disease. The dosage units were administered in the form of tablets for sucking, between meals, containing 0.00015 g of Colostrinin™ defined in Examples I and III. Firstly, 1 tablet was used daily for a period of 3 weeks, then therapy was interrupted for 2-4 weeks and treatment was repeated, administering 2 tablets daily for 3 weeks. It was found that Colostrinin™ treatment induced a state of hyporeactivity or tolerance. This is manifested by an inability to synthesise IFN and also tumour necrosis factor TNF-α. This phenomenon permits quantitative measurements of the action of active agent.

After cessation of administration of these drugs the state of tolerance reverses spontaneously. The state of temporary tolerance to the Colostrinin™ is a result of the involvement of cytokines produced by Th1 and Th2 lymphocytes and helper cells such as monocytes, macrophages, dendritic cells, and endothelial cells and their products. As a result, improvement of contact and uplift of mood were observed in patients with Alzheimer's disease.

Fig. 1 illustrates the appearance and spontaneous disappearance of a state of hyporeactivity (partial tolerance) to induction of gamma interferon (IFN-γ) in a female patient (J.M.) with Alzheimer's disease, who received Colostrinin™ in 100-μg tablets every other day for three weeks. This was followed by a 3-week pause in treatment (during the pause, the tolerance to the inducer returns to normal). Blood samples for investigating stimulation of IFN-γ by Colostrinin™ and control inducers (PHA - 10 μg/ml) were taken every week. The method of performing the tests for induction of cytokines and their quantitative determination were described in previous sections.

The results of determinations of levels of induced IFN-γ showed that hyporeactivity appears as early as during the first week of taking Colostrinin™ (100 μg/tablet every other day) and is maximum in the third week of treatment. Reversal of the state of tolerance to induction of IFN-γ occurred spontaneously in a period of 3 weeks of a pause in treatment (i.e. in

the sixth week after commencement of treatment). Moreover, this chart shows that hyporeactivity that is "specific" with respect to sheep Colostrinin™ (OvCal) is still maintained in the sixth week of observation, whereas hyporeactivity to PHA had disappeared completely.

Example X

The method of treatment of disorders of the central nervous system was investigated on a group of volunteer patients in the early stages of Alzheimer's disease. The dosage units were administered in the form of tablets for sucking, between meals, containing 0.00015 g of NP defined in Example II. Firstly, 1 tablet was used daily for a period of 3 weeks, then therapy was interrupted for 3 weeks and treatment was repeated, administering 2 tablets daily for 3 weeks. It was found that NP treatment induced a state of hyporeactivity or tolerance. This is manifested by an inability to synthesise IFN and also tumour necrosis factor TNF-α. This phenomenon permits quantitative measurements of the action of active agent.

After cessation of administration of these drugs the state of tolerance reverses spontaneously. The state of temporary tolerance to the NP is a result of the involvement of cytokines produced by Th1 and Th2 lymphocytes and helper cells such as monocytes, macrophages, dendritic cells, and endothelial cells and their products. As a result, improvement of contact and uplift of mood were observed in patients with Alzheimer's disease.

Claims

1. Colostrinin for use as a medicament.

2. Colostrinin for use as a medicament for humans.

3. Colostrinin for use in the treatment of chronic disorders of the central nervous system.

4. Colostrinin according to claim 3, for use in the treatment of neurological disorders and/or mental disorders.

5. Colostrinin according to claim 3, for use in the treatment of dementia and/or neurodegenerative diseases.

6. Colostrinin according to claim 3, for use in the treatment of Alzheimer's disease and/or motor neurone disease.

7. Colostrinin according to claim 3, for use in the treatment of psychosis and/or neurosis.

8. Colostrinin for use in the treatment of chronic disorders of the immune system.

9. Colostrinin for use in the treatment of chronic disorders of the immune system in humans.

10. Colostrinin according to claim 9 or 10, for use in the treatment of diseases with a bacterial and viral aetiology, and/or for use in the treatment of acquired immunological deficiencies.

11. Colostrinin according to claim 9, 10 or 11, for use in the treatment of chronic bacterial and/or viral infections.

12. Colostrinin according to any preceding claim, wherein said Colostrinin is derived from a non-ovine source.

13. The use of Colostrinin in the manufacture of a medicament for the treatment of chronic disorders of the central nervous system.

14. The use according to claim 13, for the treatment of neurological disorders and/or mental disorders.

15. The use according to claim 14, for the treatment of dementia and/or neurodegenerative diseases.

16. The use according to claim 14, for the treatment of for use of Alzheimer's disease and/or motor neurone disease.

17. The use according to claim 14, for the treatment of psychosis and/or neurosis.

18. The use of Colostrinin in the manufacture of a medicament for the treatment of chronic disorders of the immune system.

19. The use of Colostrinin in the manufacture of a medicament for the treatment of chronic disorders of the immune system in humans.

20. The use according to claim 18 or 19 , for the treatment of diseases with a bacterial and viral aetiology, and/or for use in the treatment of acquired immunological deficiencies.

21. The use according to claim 18, 19 or 20, for the treatment of bacterial and/or viral infections.

22. The use according to any one of claims 18 to 21, wherein said Colostrinin is non-ovine Colostrinin.

23. A method of treating disorders of the central nervous system and/or of the immune system using Colostrinin.

24. A method of treating disorders of the central nervous system and/or of the immune system, comprising administering a predetermined amount of a composition containing Colostrinin to a patient for a predetermined period of time.

25. A method according to claim 24, wherein the patient is a human patient.

26. A method according to claim 23, 24 or 25, wherein the Colostrinin is non-ovine Colostrinin.

27. A method according to any one of claims 23 to 26, comprising wherein said predetermined amount of Colostrinin is in the range of about 25 to 2000 micrograms.

28. A method according to claim 27, comprising a cycle of administering 25 to 2000 micrograms of Colostrinin each day to a patient for a first period, followed by a second period when no Colostrinin is administered.

29. A method according to claim 28, wherein the first period is in the range of about 2 to 4 weeks, and the second period is in the range of about 2 to

5 weeks.

30. A method according to claim 28 or 29, wherein the cycle is repeated at least once.

31. A pharmaceutical composition comprising a preselected amount of Colostrinin in combination with a physiologically acceptable carrier.

32. A composition according to claim 31, wherein the Colostrinin is non-ovine Colostrinin.

33. A composition according to claim 31 or 32, in a form suitable for injection.

34. A composition according to claim 31 or 32, in a form suitable for absorption through the mucosa of the oral/nasopharyngeal cavity and/or in a form suitable for absorption in the alimentary canal.

35. A composition according to any one of claims 31 to 34, in the form of a tablet, lozenge, gel, patch or plaster.

36. A composition according to any one of claims 31 to 35, comprising 25 to 1000 micrograms of Colostrinin.

37. A composition according to any one of claims 31 to 35 comprising 50 to 100 micrograms of Colostrinin.

39. The use of Colostrinin as a dietary supplement.

40. The use of Colostrinin as a dietary supplement for babies, small

children, adults who have been subjected to chemotherapy and/or adults who have suffered from cahexia, or weight loss due to chronic disease.

41. A dietary supplement comprising an orally ingestible combination of Colostrinin in combination with a physiologically acceptable carrier.

42. Colostrinin for use in the stimulation and/or modulation of the immune system of mammals.

43. Colostrinin for use in the stimulation and/or modulation of the immune system of humans.

44. The use of Colostrinin in the manufacture of a medicament for use in the stimulation and/or modulation of the immune system of mammals.

44. The use of Colostrinin in the manufacture of a medicament for use in the stimulation and/or modulation of the immune system of humans.

45. Colostrinin for use as a prophylactic medicament.

46. Colostrinin for use as a prophylactic medicament for humans, to prevent or inhibit the development of Alzheimer's disease.

47. The use of Colostrinin in the manufacture of a prophylactic medicament for humans, to prevent or inhibit the development of Alzheimer's disease.

48. A method of preparing Colostrinin from mammalian colostrum, comprising

(1) Removing lipids and the majority of proteins from the colostrum;

(2) separating Colostrinin bound to immunoglobulin from the colostrum; and

(3) Separating the Colostrinin from the immunoglobulin, and purifying the Colostrinin.

49. A method according to claim 48, wherein in step (1) the lipids are removed by centrifuging and the proteins are removed by pH lowering; in step (2) the Colostrinin bound to immunoglobulin is removed from the colostrum by processing the fraction formed after the removal of lipids and proteins by ion exchange chromatography, eluting with phosphate buffered saline and collecting a fraction containing Colostrinin bound to immunoglobulin; and step (3) comprises separating Colostrinin from the immunoglobulin by sieving chromatography, and further purifying the Colostrinin by de-salting the fraction below 30,000 Daltons molecular weight and introducing antibodies to immunoglobulins and thereby remove this class of proteins to obtain the final product.

50. A method of making a pharmaceutical composition comprising combining Colostrinin with a physiologically acceptable carrier, and forming said mixture into a form in which it can be administered to a patient.

51. A nanopeptide having the composition and amino acid sequence Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro for use as a medicament.

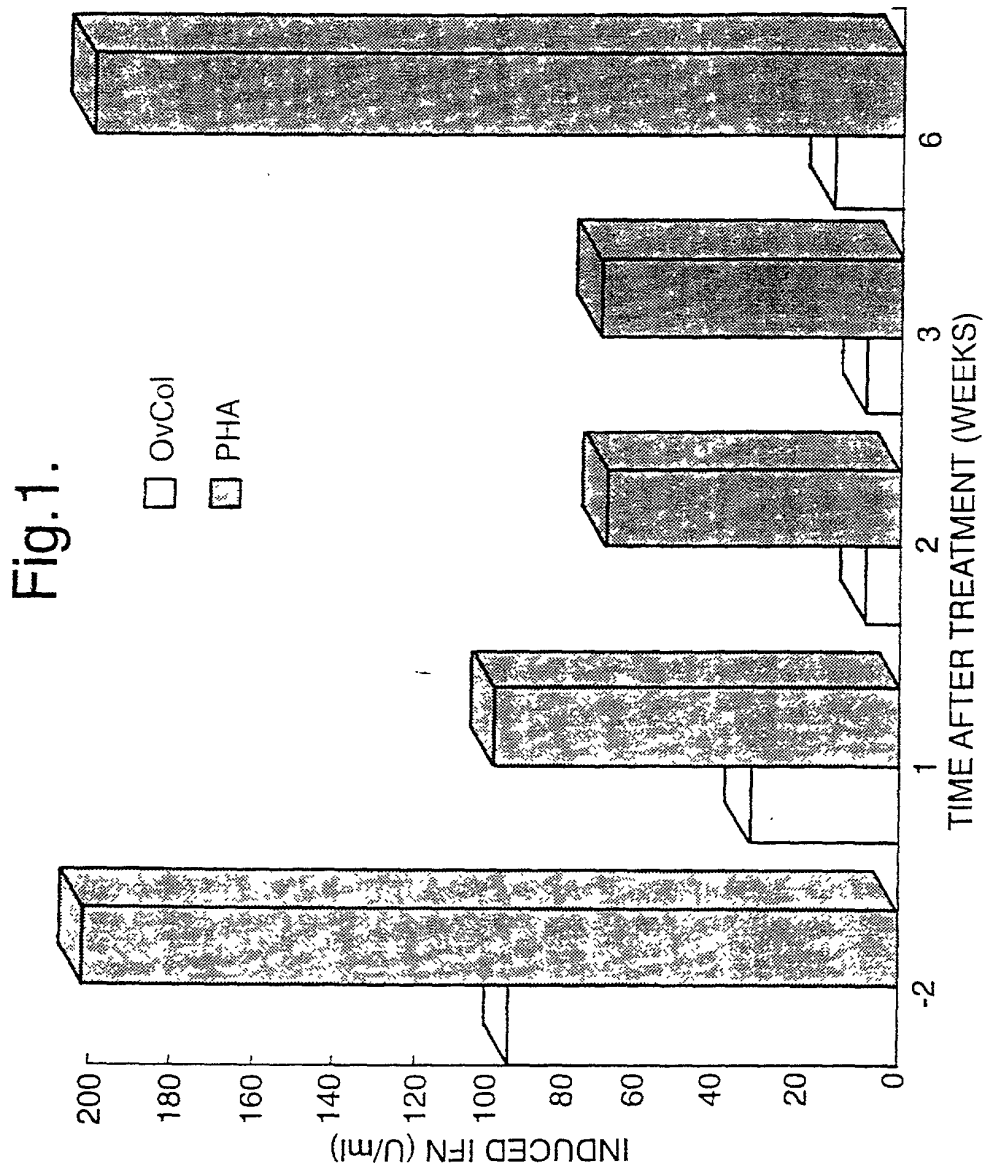
52. The use of a nanopeptide having the composition and amino acid sequence Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro in the manufacture of a medicament for treating chronic disorders of the immune system in humans.

53. The use of a nanopeptide having the composition and amino acid sequence Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro in the manufacture of a medicament for treating chronic disorders of the central nervous system.

54. A pharmaceutical composition comprising a nanopeptide having the composition and amino acid sequence Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro in combination with a physiologically acceptable carrier.

55. A method of making a pharmaceutical composition comprising combining a nanopeptide having the composition and amino acid sequence Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro with a physiologically acceptable carrier, and forming said mixture into a form in which it can be administered to a patient.

Fig.1.


OvCol
PHA
INDUCED IFN (U/ml)
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TIME AFTER TREATMENT (WEEKS)

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	AAT-11612
First Named Inventor	Marin Janusz
COMPLETE IF KNOWN	
Application Number	09 / 269,845
Filing Date	March 31, 1999
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

COLOSTRININ, AND USES THEREOF

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 03/31/99 as United States Application Number or PCT International Application Number 09/269,845 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
PCT/GB97/02721	PCT	10/03/97	☐	☐	☒
P.316416	Poland	10/03/96	☐	☐	☒
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (If applicable)
PCT/GB97/02721	10/03/97	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → Place Customer Number Bar Code Label here
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Name	Registration Number	Name	Registration Number
Kenneth A. Clark	32,119		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

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Post Office Address							
City	Wroclaw	State		ZIP	53-124	Country	Poland

☒ Additional Inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname
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Inventor's Signature	J Lisowski					Date	1.09.1999
Residence: City	Wroclaw PLX	State		Country	Poland	Citizenship	Polish
Post Office Address	Wyb. Pasteura 17a/18						
Post Office Address							
City	Wroclaw	State		ZIP	50-367	Country	Poland

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname
Mieczyslaw Inglot, heir and executor for	Anna Dubowska-Inglot, deceased

Inventor's Signature	M Inglot					Date	1.09.1999
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Post Office Address							
City	Wroclaw	State		ZIP	53-651	Country	Poland

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle [if any])	Family Name or Surname

Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
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City		State		ZIP		Country	

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